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Sexual Maturation in Female Rats: Time-Related Changes in the Isoelectric Focusing Pattern of Anterior Pituitary Follicle-Stimulating Hormone[1]

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ABSTRACT

Anterior pituitary glands (AP) were obtained from female rats at 5, 15, 18, 21 and 29 days of age, at the time of vaginal opening (VO) and during adulthood on proestrus. The multiple species of follicle-stimulating hormone (FSH) present within the AP were separated by the technique of polyacrylamide gel isoelectric focusing (PAG-IEF) and measured with the NIAMDD rat FSH radioimmunoassay kit. AP's obtained from immature female rats prior to VO contained elevated levels of total FSH as well as all of the species of AP FSH observed in adult rats (and hamsters). However, the majority of the FSH immunoactivity migrated to the most acidic portion of the gel (isoelectric point [pI] value=4.2–3.8). At the time of VO and during adulthood, a decrease in total AP FSH was observed. In addition, a shift in the relative proportions of certain FSH species occurred. The AP's of adult animals contained relatively greater amounts of more basic (pI values 6.0–5.0) forms of FSH compared with immature animals. When each of the AP FSH species isolated from adult animals was tested in a radioligand receptor assay, the most acidic (pI=4.2–3.8) failed to interact with the receptor preparation, while those with pI values from 6 to 4.7 were able to compete with [^{125}I]-labeled FSH for receptor binding in a parallel fashion. Thus, the observed shift in the PAG-IEF FSH profiles to more basic (and biologically active) forms may represent a change in the composition of AP FSH that serves an important role in the maturation process leading to ovulatory cyclicity.

INTRODUCTION

We have recently described the existence of multiple species of follicle-stimulating hormone (FSH) present within the anterior pituitary glands of adult female (Chappel, 1981; Chappel et al., 1982) and male (Ulloa-Aguirre and Chappel, 1982a) hamsters with the techniques of concanavalin A chromatography and polyacrylamide gel isoelectric focusing (PAG-IEF). In those studies, we observed that the relative amounts of some of those species of FSH changed depending upon the endocrine status of the donor. Not only were differences observed in the absolute amounts of each of the FSH species, but those species also exhibited differences in their radioreceptor:radioimmunoassayable content. From those studies, we have concluded that the surrounding hormonal milieu influences not only the biosynthesis and secretion rates of those species of FSH, but also their receptor binding activity.

The transition from sexual immaturity to adulthood represents a period of rapid change in serum hormone levels (Dohler and Wuttke, 1974, 1975; Ramaley, 1979), and hypothalamic gonadotropin releasing hormone (GnRH) content (Araki et al., 1975). Drastic changes in pituitary content and serum levels of FSH have been observed in developing female rats (Dohler et al., 1977; Watanabe and McCann, 1972). Changes in FSH secretion appear to play a role in the maturation of the hypothalamic-pituitary-gonadal axis (Uilenbroek et al., 1976). There-

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fore, we performed the following studies to examine whether the heterogeneous nature of pituitary FSH is observed during sexual development in the immature female rat and have correlated these changes with the animal's age and other endocrine parameters.

MATERIALS AND METHODS

Pregnant female Sprague-Dawley rats were obtained from Sasco Labs. (Omaha, NE) and placed in individual stainless steel cages. The day of birth was designated as Day 1 of life. Female pups were decapitated (at 0800 h) at 5, 15, 18, 21 or 29 days of age in groups of at least 10. Additional groups of animals (n=10) were decapitated at 0800 h on the day of vaginal opening or during adulthood on the morning (0800 h) and afternoon (1730 h) on the day of proestrus. Trunk blood was obtained from each individual except the 5-day-old animals. In that case, trunk blood was pooled from groups of 5-day-olds due to the small volume obtained. Blood was allowed to clot and serum samples were stored at −20°C until the day of the radioimmunoassay (RIA). The anterior pituitary gland was dissected free from the posterior pituitary, weighed and homogenized with ten strokes of a teflon pestle in 1 ml of phosphate-buffered saline (PBS: 0.05 M phosphate; 0.15 M NaCl; pH=7; 4°C). The homogenate was centrifuged at 1000 × *g* for 30 min and the supernatant was removed and stored at −20°C until the day of the FSH RIA or polyacrylamide gel isoelectric focusing (see below). In addition, the hypothalamus was removed from each animal (anterior border: optic chiasm; posterior border: mammillary bodies; lateral border: hypothalamic fissures; dorsal border: 2 mm from the ventral surface) and homogenized in 1 ml of 0.1 N HCl. Each homogenate was centrifuged as above and the supernatant was stored at −20°C until the day of the GnRH RIA. Body, anterior pituitary, ovarian and uterine weights were also recorded.

A portion of each pituitary extract was removed and measured for FSH and luteinizing hormone (LH) content by RIA. The proteins present within each extract were precipitated by the following method: to each extract was added 100 μl of a 2% (w/v) solution of bovine serum albumin (BSA; Fraction V; Sigma Chemical Co., St. Louis, MO) in PBS. Immediately thereafter, 3 ml of an ethanol-acetic acid solution (100: 1 v/v; pH=3) was added to each sample. Following vortexing, each sample was incubated on ice for 30 min (1000 × *g*) and the precipitates formed were dried under a stream of nitrogen. The resulting precipitate was solubilized in 50 μl of PBS and the proteins present within the solution were separated by the technique of polyacrylamide gel isoelectric focusing (PAG-IEF) as described previously (Chappel et al., 1982). Briefly, preformed PAG-IEF plates (pH range= 6.5−4.0) were purchased from LKB Instruments, Inc. (Bethesda, MD). Samples were separated according to the instructions provided by the manufacturer. At the end of the 2.0- to 2.5-h focusing period, the pH of each sample was determined with a surface electrode (Microelectrodes, Inc., Londonderry, NH), each sample lane was sliced into twenty-five 3-mm sections and proteins focused within each gel slice were eluted by incubation in 400 μl PBS plus 0.1% gelatin for 12−18 h. Each gel slice eluent was then measured for FSH content by RIA.

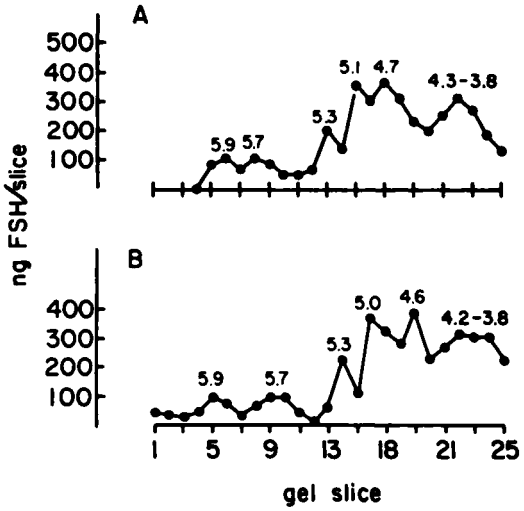

FIG. 1. The isoelectric focusing pattern of FSH present within NIAMDD rat FSH-RP-1 before *(A)* and after *(B)* acid-ethanol precipitation (See *Materials and Methods* for details). The numbers above each peak in FSH immunoactivity in this and subsequent figures represents the isoelectric point of that peak.

To ascertain whether the acid-ethanol precipitation of AP proteins described above affected the isoelectric focusing pattern of FSH, a small amount of NIAMDD rat FSH-RP-1 was solubilized in PBS. The sample was divided in half and one half was applied directly to the isoelectric focusing gel. The other half was treated as described with BSA and acid-ethanol, precipitated, solubilized and then subjected to PAG-IEF. The resulting PAG-IEF profiles were compared (Fig. 1).

As depicted in Fig. 1, precipitation of FSH present within NIAMDD rat FSH-RP-1 with acid-ethanol failed to influence the PAG-IEF profile of FSH. From these results, it was concluded that this method could be employed to concentrate pituitary FSH for analysis by PAG-IEF. As observed in other studies (Chappel et al., 1982), FSH activity that migrated to the most acidic portion of the gel was not sharply focused, and exhibited a pI value between 4.3−3.8.

Protein Hormone Radioimmunoassays

FSH and LH content of pituitary and serum samples were determined by RIA kits generously provided by the NIAMDD. Iodinated LH and FSH were prepared by the enzymatic methods described by Bex and Corbin (1982) and were further purified by concanavalin A chromatography as described by Dufau et al. (1972). The specific activity for both hormone preparations was approximately 100 μCi/μg. Within and between assay variabilities for the LH and FSH were 6.5% and 12.1% and 8.4% and 10.2%, respectively. The sensitivities of the LH and FSH RIA's were 2 ng and 15 ng, respectively. All values for

the LH and FSH RIA's are expressed in terms of NIAMDD rat RP-1 standard (FSH biopotency=2.1 × NIH FSH–S1; LH biopotency=0.03 × NIH LH–S1). GnRH activity within hypothalamic supernatants was determined by a RIA as described by Nett et al. (1973) using the R-42 antisera provided by Dr. T. Nett. Iodinated GnRH was prepared according to the methods described by Wagner et al. (1979). Purified GnRH (synthetic) used for labeled antigen, as well as the standard curve, was purchased from Calbiochem (La Jolla, CA). The within and between assay variabilities for this RIA system were 6.2% and 13.6%, respectively. The sensitivity of the assay was 1 pg/tube.

FSH Radioreceptor Assay

Following separation by PAG-IEF, each of the six species of FSH within pituitaries of adult female rats were tested for receptor binding activity. PAG-IEF gel eluents containing the highest concentration of each of the FSH species (as determined by RIA) were tested at four dose levels for the ability to displace [^{125}I]-FSH from a FSH receptor preparation. FSH receptors were prepared from 28-day-old male rat testes as described by Bhalla and Reichert (1974). The details of the radioreceptor assay are described elsewhere (Ulloa-Aguirre and Chappel, 1982a). The sensitivity of the receptor assay used for this study was approximately 20–40 ng/tube. The [^{125}I]-FSH used for the binding study was prepared as described above, however, the specific activity was approximately 40 μCi/μg.

Statistical differences (α=0.05) between groups were determined by one-way analysis of variance and Student's *t* test for unpaired samples.

RESULTS

Shown in Table 1 are the age-related changes in body, pituitary, ovarian and uterine weights in female rats used in the present study. These data are similar to those reported by others (for review see Ramaley, 1979) and are included so that the reader may compare the rate of maturation with other strains.

As depicted in Fig. 2A, measurement of serum levels of FSH in maturing female rats disclosed a significant ($P<0.01$) elevation at 15 days of age (1515 ± 163 ng/ml) compared with 5-day-old animals. Thereafter, serum FSH concentrations declined until the day of vaginal opening, and the first ovulation, at which time a significant elevation was observed (337 ± 71 ng/ml) in the morning compared with animals sacrificed at 29 days of age (183 ± 25 ng/ml; $P<0.05$). This elevation in serum FSH levels in animals at the time of vaginal opening is probably the well-described secondary rise in FSH that follows proestrus. Finally, as expected, a significant elevation in serum FSH levels was observed during the late afternoon of proestrus in adult animals compared with those

TABLE 1. Age-related changes in organ weights.

Age days	Number of animals	Body weight (g)	Pituitary		Ovaries		Uterus	
			mg	mg/100 g BW	mg	mg/100 BW	mg	mg/100 BW
5	35	11.3 ± 0.2	0.9 ± 0.1	7.7 ± 0.6	–	–	–	–
15	15	27.1 ± 0.7	1.4 ± 0.04	5.0 ± 0.1	4.2 ± 0.3	15.6 ± 1.1	17.6 ± 0.9	64.5 ± 2.8
18	15	32.5 ± 0.9	1.5 ± 0.07	4.4 ± 0.1	6.5 ± 0.3	19.9 ± 0.8	17.9 ± 0.6	55.7 ± 2.3
21	10	39.0 ± 0.9	1.6 ± 0.07	4.2 ± 0.2	8.9 ± 0.3	23.1 ± 1.1	26.1 ± 1.3	67.2 ± 3.4
29	10	81.3 ± 2.4	2.8 ± 0.1	3.4 ± 0.1	13.7 ± 1.7	19.0 ± 0.9	54.3 ± 3.2	66.8 ± 3.7
VO (38.1 ± 1.1 days)	10	129.3 ± 5.4	4.9 ± 0.2	3.8 ± 0.1	26.5 ± 2.8	19.7 ± 1.9	201.8 ± 21.8	156.9 ± 17.8
Adult (proestrous morning)	10	267.1 ± 6.7	9.6 ± 0.7	3.6 ± 0.3	86.6 ± 5.1	33.6 ± 1.4	508.8 ± 56.6	191.8 ± 22.6

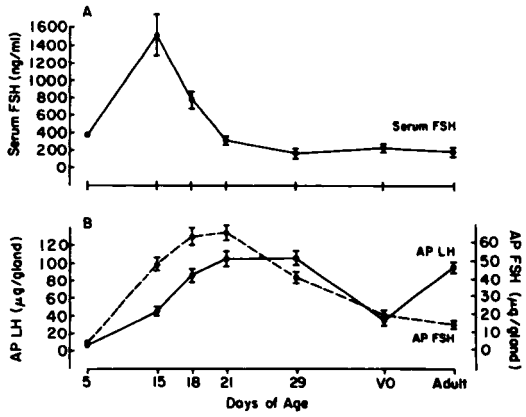

FIG. 2A. Serum levels of FSH in immature female rats before vaginal opening *(VO)*, on the day of VO and during proestrous morning of adulthood. Each point represents the mean ± SEM for at least five individual animals except 5-day-old aminals.

FIG. 2B. Content of LH and FSH within the anterior pituitary glands of immature female rats before vaginal opening *(VO)* on the day of VO and on the morning of proestrus in adults.

killed during the morning (488 ± 50 ng/ml vs. 191 ± 20 ng/ml; $P<0.05$).

Due to pulsatile releases (Frawley and Hendricks, 1979) the variation in serum LH levels within a group of animals at the time of pituitary collection was great. Therefore, no significant differences were observed between groups (data not shown). However, a significant ($P<0.001$) elevation in serum LH levels was observed during the late afternoon of proestrus in adult animals (2390 ± 593 ng/ml) compared with adults killed during the morning of proestrus (15 ± 2.5 ng/ml).

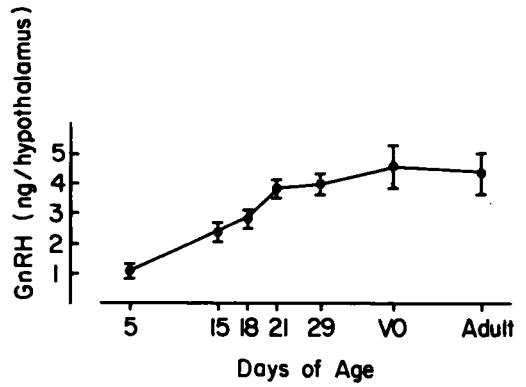

FIG. 3. The content of hypothalamic GnRH on selected days in immature female rats, on the morning of vaginal opening *(VO)* and on proestrous morning.

As shown in Fig. 2B, pituitary FSH content rose dramatically from Day 5 to Day 15 of life and reached maximal levels (54.64 ± 4.7 μg/gland) by Day 18. These levels remained elevated by Day 21 and began to decline by Day 29. Thereafter, FSH levels within the pituitary gland reached lowest values by adulthood on proestrous morning (12.87 ± 1.47 μg/gland). As observed with FSH, pituitary LH content was maximal during Days 18–29 of life (106 ± 10.5 μg/gland). These values declined to a nadir on the day of vaginal opening (34.5 ± 10.9 μg/gland) and were significantly ($P<0.01$) elevated again by adulthood on proestrous morning (92.5 ± 7.0 μg/gland).

Hypothalamic GnRH (Fig. 3) rose steadily to a maximum level observed by Day 21 of life (3807 ± 192 pg/hypothalamus) and remained at that approximate concentration into adulthood.

When a portion of the pituitary obtained from each animal prior to vaginal opening was subjected to PAG-IEF, seven distinct species of FSH were detected by RIA. Six of those species had pI similar to those observed when NIAMDD rat FSH-RP-1 was tested. The seventh species of FSH (pI=4.2) appears as a shoulder to the most acidic peak and was not clearly defined when the FSH RP-1 was tested. This FSH form was not a distinct peak in adult animals (Fig. 5). The isoelectric focusing pattern of FSH obtained from pituitaries of immature female rats appeared to be different than that observed with the NIAMDD FSH RP-1. To quantitate these differences, each PAG-IEF FSH profile obtained from each animal was divided into five regions and the amount of FSH present within each was deterined. The pI boundries of each area were as follows: *Area I*=6.0–5.5; *Area II*=5.49–5.0; *Area III*=4.99–4.5; *Area IV*=4.49–4.0; and *Area V*=3.99–3.49.

As shown in Table 2 and Fig. 4, when animals at ages prior to vaginal opening were compared, no significant changes with age were observed in the relative proportions of FSH present within any of the five areas. Clearly, Area V contained the greatest proportion of FSH activity. Since the relative amounts of FSH focusing in each area of the gel did not vary with age in animals tested prior to vaginal opening, the results from each group were combined and treated as one.

Shown in Fig. 5 are representative PAG-IEF profiles obtained from animals at the time of

TABLE 2. Distribution of AP FSH across a pH gradient: changes with age.

Age (days)	Area I (pI-6–5.5)	Area II (pI-5.49–5.0)	Area III (pI=4.9–4.5)	Area IV (pI-4.49–4.0)	Area V (pI=3.9–3.49)
5	11.4[a]	16.5	14.7	14.9	40.5
15	5.0	13.1	15.3	21.4	40.1
18	6.5	11.5	14.9	23.8	41.9
21	10.3	9.6	16.7	17.5	45.0
29	8.9	14.7	14.5	16.9	43.2
$\bar{X}$ ± SEM (n=25)	8.5 ± 0.84[b]	13.7 ± 0.88	16.7 ± 0.95	19.9 ± 1.0	41.4 ± 1.6
VO	19.7	20.6	18.5	15.5	30.9
Adult	19.5	17.8	14.4	17.5	30.2
$\bar{X}$ ± SEM (n=10)	19.4 ± 2.8[cd]	18.1 ± 1.2[e]	16.3 ± 1.8	17.4 ± 0.84	30.6 ± 1.2[d]

[a]Each determination represents the percentage of AP FSH that focused in the corresponding pH range and is the mean obtained for at least five AP samples collected for animals at that age.

[b]Mean percentage ± SEM of FSH focusing in a pH range when individual proportions from all animals (ages 5–29) are combined and treated as one group.

[c]Mean percentage ± SEM of FSH focusing in a pH range when individual proportions from all animals (at VO or Adult) are combined and treated as one group.

[d]Significantly different from value obtained from immature (ages 5–29 days) group ($P<0.01$).

[e]Significantly different from immature (ages 5–29 days) group ($P<0.05$).

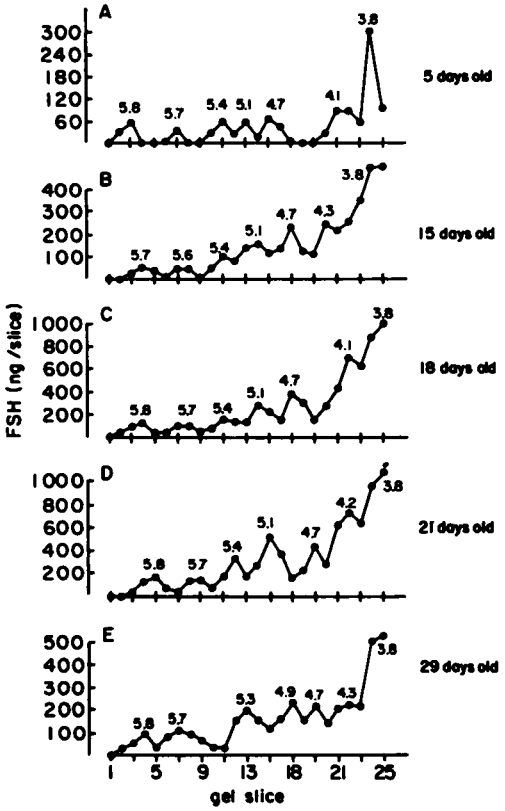

FIG. 4. The polyacrylamide gel isoelectric focusing pattern of immunoassayable FSH present within immature female rats prior to the day of vaginal opening.

vaginal opening (38.1 ± 1.1 days) and adulthood (during proestrous morning). Note the presence of the seven species of FSH within the pituitary of female rats at vaginal opening. However, at adulthood, only six distinct species of FSH were observed. The seventh species (pI value = 4.2) was not sharply focused in adults and may explain the broad range of FSH activity observed in the portion of the gel. These species of pituitary FSH exhibited pI values similar to that observed with NIAMDD-rat-FSH-RP-1. The amount of FSH activity present in each area of the gel was calculated and statistical analyses were performed (Table 2). No change in the relative proportions of FSH in that area or any of the other areas of the gel were observed compared with animals at the time of vaginal opening (Table 2). Therefore, all animals in both groups (vaginal opening and adult) were combined and treated as a single population.

Thus, when the two groups of animals (prior to and after vaginal opening) were compared, the following differences were observed: the relative proportions of FSH focusing in Area V of the gel declined significantly ($P<0.01$) after vaginal opening. Concomitantly, the relative proportions of AP FSH activity present within Areas I and II increased ($P<0.01$ and $P<0.05$, respectively). Each of the species of FSH obtained from the anterior pituitary glands of adult female rats was tested for receptor binding activity using male rat FSH receptors present within a seminiferous tubule homogenate. As shown in Fig. 6, the species of FSH with pI values of 5.9, 5.7, 5.3, 5.1 and 4.7 (which focused in Areas I–III) were capable of displacing, in a parallel fashion, [^{125}I] -labeled FSH from a biological receptor preparation. The FSH species with pI=4.3–3.8 (which focused in Areas IV and V) failed to exhibit this receptor binding activity.

DISCUSSION

It is known that an immature (anovulatory) female rat may be induced to ovulate with an injection of a pharmacological dose of estradiol as early as 16 days of life (Andrews et al., 1981). However, naturally occurring ovulation is not observed until many days later. The mechanisms that underlie this progression to ovulatory cyclicity have been the subject of a great deal of investigation. In the female rodent, it appears that the presence of α-fetoprotein plays a significant role in the animals' ability to recognize circulating estradiol levels

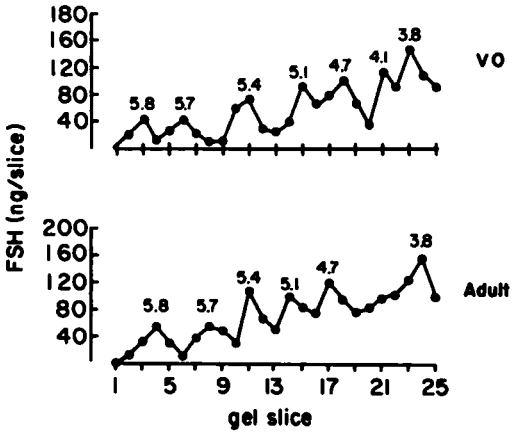

FIG. 5. The polyacrylamide gel isoelectric focusing pattern of immunoassayable FSH present within animals on the day of vaginal opening and during proestrous morning.

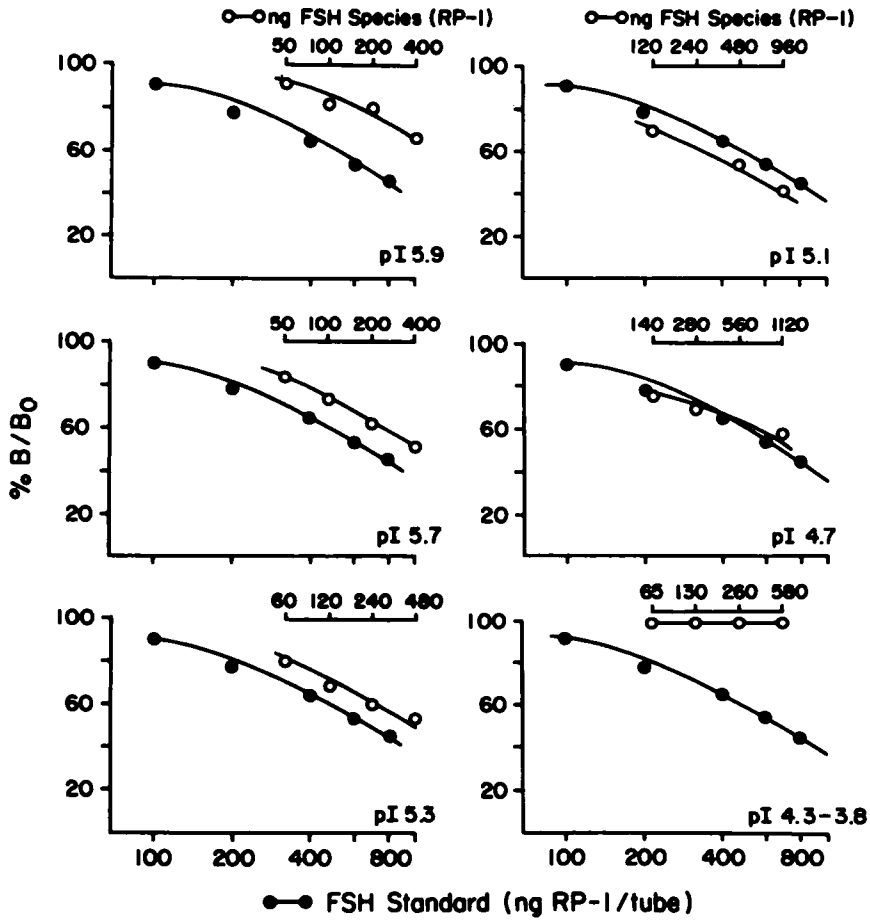

FIG. 6. The receptor binding capacity of each AP FSH species (denoted by isoelectric point [*pI*]) obtained from adult female rats. Note the ability of increasing amounts (as quantitated by radioimmunoassay) of each of the first five species of FSH to displace [^{125}I]-labeled FSH from a biological receptor preparation in a fashion parallel to NIAMDD rat FSH RP-1. The most acidic FSH species (pI value=4.3–3.8) was ineffective in this regard.

and therefore in the timing of the onset of puberty (Andrews et al., 1981). This binding protein (rather than the attainment of adult levels of GnRH within the hypothalamus or the ability of the anterior pituitary gland to respond to GnRH) appears to be the "rate-limiting step" in the maturation process. Adult levels of GnRH are present within that gland many days before the first ovulation (Araki et al., 1975; Ojeda et al., 1976; the present study). The anterior pituitary gland of juvenile female rats contains receptors for GnRH (Dalkin, et al., 1981), is exquisitely sensitive to GnRH challenges (Debeljuk et al., 1972; Ojeda et al., 1977) and responds to castration by hypersecretion of pituitary gonadotropins (Goldman et al., 1971; Andrews et al., 1981). As shown by others and confirmed in the present study, the pituitary of immature animals contains large amounts of FSH. A portion of this hormone is biologically active as demonstrated by Ojeda and Jameson (1977). This finding has also been observed experimentally as the inhibition of FSH secretion on Day 15 of life disrupts the natural process of folliculogenesis in the female rat (Uilenbroek et al., 1976). Thus, it appears from the above studies that the anterior pituitary gland of the female rat is capable of functioning quite early in life. Is the pituitary gland fully mature at that time?

One possible means to examine this question is to measure the subpopulations of FSH

present within pituitary glands of developing female rats and compare relative proportions with those obtained from mature, cycling animals.

We have recently demonstrated by polyacrylamide gel isoelectric focusing (PAG-IEF), the presence of multiple species of FSH within the anterior pituitary gland of female and male hamsters (Chappel et al., 1982; Ulloa-Aguirre and Chappel, 1982a). The relative proportions of these species change depending upon the endocrine status of the pituitary donor.

The present study extends our earlier findings by demonstrating the existence of multiple species of FSH present within the rat pituitary that are separable by the technique of PAG-IEF. At 5 days of age, the female rat pituitary contains six species of FSH with pI values similar to that observed in male and female hamsters, adult female rat and NIAMDD FSH-RP-1. In addition, a seventh species (pI value=4.2), which appears as a shoulder to the most acidic form (pI value=3.8) of pituitary FSH, is also present. This species of FSH is not sharply focused in adult animals. Clearly, the majority of the FSH present within the pituitary of female rats prior to vaginal opening migrates to the most acidic portion of the gel (Area V) and has a pI value of 4.2–3.8. Less than 10% of the FSH present within the pituitary of female rats prior to vaginal opening was detected in more basic forms (Area I). At the time of vaginal opening, the content of FSH within the pituitary gland drops precipitously. Interestingly, at the same time, a shift in the relative proportions of the species of FSH occurs. A greater percentage of pituitary FSH focuses in the more basic portions of the gel (Area I). A proportional drop in the amount of FSH focusing in the Area V is observed at that time. Thus, as the female rat develops sexually, no discernable change in the FSH subpopulation PAG-IEF profile is observed until the time of vaginal opening.

As we have previously reported in the hamster (Ulloa-Aguirre and Chappel, 1982a,b), marked differences exist in the receptor binding: radioimmunological FSH activity ratios when each of the species of female rat pituitary FSH was tested.

In the present studies, species of FSH that focused in Areas I–III of the gel (pI value 6.0–4.5) exhibited a greater ability to displace [^{125}I]-FSH from an FSH receptor preparation than did FSH that focused in Areas IV or V (pI value 4.49–3.49). In male hamsters, an excellent correlation exists between the receptor binding and biological activity of each species of FSH (Ulloa-Aguirre and Chappel, 1982b).

To date, we have observed that FSH present in the pituitary glands of adult male (unpublished observations) and female rats (the present study) exhibits an isoelectric focusing profile similar to NIAMDD rat FSH RP-1 as well as male (Ulloa-Aguirre and Chappel, 1982a,b) and female hamsters pituitary homogenates (Chappel et al., 1982). Rat and hamster FSH forms are recognized by hamster and rat FSH receptor preparations (the present study; Ulloa-Aguirre and Chappel, 1982a,b). In addition, we have found that these species of pituitary FSH obtained from rats and hamsters exhibit similar radioreceptor:radioimmunoassay ratios. Finally, both hamster and rat FSH preparations are active in an FSH bioassay that employs rat granulosa cells (Ulloa-Aguirre and Chappel, 1982b). Combined, these data suggest that the multiple forms of FSH obtained from rats and hamsters are quite similar biochemically, immunologically and biologically.

The present studies suggest that small but significant amounts of biologically active FSH are present in 5- to 29-day-old female rat pituitaries (Areas I and II). However, a much greater proportion is biologically inactive (Area V). These observations confirm and extend the findings of Ojeda and Jameson (1977) who have demonstrated that the pituitary gland of animals at 12 days of age contain biologically active forms of FSH. These authors found no change in the bioassay/radioimmunoassay ratio for immature rat pituitary FSH at 12 or 27 days of age. This observation parallels our inability to discriminate FSH PAG-IEF profiles in immature rats at any age prior to vaginal opening.

It appears that biologically active forms of FSH are present within the pituitary at the earliest time tested: 5 days of age.

At the time of vaginal opening, a decrease in total pituitary FSH, as well as a shift in the relative proportions of inactive/active species of FSH, is observed. A similar shift in biologically active forms of LH has been observed at puberty in human males and females (Lucky et al., 1980; Reiter et al., 1982).

These studies suggest that, at the time of vaginal opening, an endocrine event occurs that stimulates the production of greater amounts of biologically active forms of FSH. The basis of

the species microheterogeneity in hamster (Chappel et al., 1982) and monkey (Peckham and Knobil, 1976) FSH appears to be the incorporation of sialic acid residues into the protein hormone. Changes in sialic acid residues within the FSH molecule affects the plasma half-life (Peckham and Knobil, 1976) and receptor binding activity (Moore and Ward, 1980); thus, activation of pituitary enzymes to perform this function may be involved. Although the stimulus for this event is unknown, decreases in hypothalamic GnRH (and therefore increased GnRH secretion) have been reported at the time of vaginal opening (Araki et al., 1975; Sarkar and Fink, 1979). Our data does not show a depletion of hypothalamic GnRH on the morning of vaginal opening. Perhaps if the animals were killed late during the afternoon of the prior day (proestrus), significant differences would have been apparent. GnRH is capable of increasing the carbohydrate incorporation into the rodent LH molecule (Liu and Jackson, 1979) and gonadotropins released by the AP after GnRH treatment are more biologically active (Mukhopadhyay et al., 1979). Therefore, this neurohormone may be a causative factor in the shift in PAG-IEF profile at vaginal opening. Further studies are currently underway to more critically examine the intracellular mechanisms responsible for this phenomenon in the female rat.

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